THE EFFECTS OF TEMPERATURE, pH, AND MEDIUM ON HYPHAL FUSIONS IN THE THELEPHORACEAE

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By

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1. INTRODUCTION

Decays of the heartwood of living coniferous and deciduous trees are generally regarded as the most important single type of disease in North American forests. The loss of timber caused by the action of forest insects and disease organisms in Canada has been estimated at 500 million cubic feet annually, approximately one seventh of the total annual depletion from all causes and more than twice the annual loss due to fire. Decay fungi are responsible for a goodly share of this lost wood volume and have, as a result, been the subject of much of the research undertaken by forest pathologists.

One of the problems faced by the research worker concerned with decay losses is the identification of these causal fungi. All cultural identification work must ultimately rest on a comparison between the unknown isolate and cultures made from sporophores which have been definitely identified. These comparisons are usually based on detailed descriptions of the cultures. Some aspects of a physiological method of comparing cultures are discussed in this paper.

Cultures of the fungi causing decay made from affected wood were reported by Long and Harsch (32) in the United States in 1918. Since that time, the widely accepted method of determining decay fungi using their characteristics when grown in pure culture has been developed. In this trend, the work of Fritz (23) was of a pioneering nature. Cartwright and Findlay (12, 13, 14) in England, Davidson, Campbell, and Vaughn (15) in the United States and Nobles (41) in Canada have developed the techniques to their present high level. The most comprehensive work along these lines is by Nobles (41), who presented detailed keys to the cultures of some 126 species

of wood rotting fungi. The method involves growing the fungus to be identified on petri plates for six weeks, and making periodic examinations both microscopic and macroscopic during this period. While the method is precise, it has several important disadvantages, as follows: it requires a relatively long time to make a positive identification, detailed descriptions of ~~unknowns~~ cultures must have been made and included in the key, and a considerable amount of experience on the part of the worker is necessary.

Recent studies by Cabral (11) in testing the postulates of Buller (7) and Vandendries (53) that vegetative hyphal fusions are a reliable criterion of species, have served to focus attention on this method as a rapid technique for the identification of wood rotting fungi in culture. Cabral demonstrated that intra-specific hyphal fusions occurred within a week to ten days under the conditions of his experiments in 19 of the 21 species of Polyporaceae tested. No inter-specific fusions are reported although they were attempted. Cabral did not make any attempt to discover the effect of various environmental factors, except culture media, on the tendency of fungi to fuse.

The objectives of the present study are outlined as follows: to determine whether dikaryotic hyphal fusions occur in the Thelephoraceae, to examine the effect of formula and pH of culture medium as well as incubation temperature on hyphal fusion formation.

2. REVIEW OF LITERATURE

2.1) Wood Rot Fungi in Culture.

Many wood destroying fungi were treated in the extensive culture

work of Brefeld (4) and scattered information on the cultural characteristics of several species is given. Descriptions of several species in culture, particularly fungi causing dry rot in buildings, were provided by Mez (38) and Falck (20). Bavendamm (1) made several contributions to the study of wood rot fungi in culture, the most noteworthy being his observation on the oxidase reaction. The oxidase exuded by many species produces a brown stain on gallic acid or tannic acid agar. This feature is useful in separating white rot fungi from brown rot fungi, the latter group producing the stain.

In the United States, the first detailed research on the culturing of wood decay fungi was by Long and Harsch (32). Definite diagnostic keys were not presented but the value of many cultural characters in identification was discussed. Snell (49) presented a diagnostic key to five important wood destroying fungi based on the microscopic and macroscopic features of the organisms and their temperature relationships when grown on malt agar.

In Canada, Fritz (23) made a valuable contribution to the cultural identification of wood rotting fungi in her detailed study of 17 major decay organisms. Fritz tested a large number of vegetable agar media and in most cases, many strains of each fungus. She concluded that two media, potato dextrose agar and plain malt agar, were the most valuable for the distinction of her fungi in culture. Fritz's work also contained reference to the temperature relations of some of the fungi she studied. However, little actual use was made of these temperature data in her identification keys. The recent study of Nobles (41) is without precedence in this field.

Many detailed papers have been presented dealing with the biology of one particular fungus. These studies frequently give data on the behavior

of the fungus in culture and descriptions of its cultural features. Their utility in cultural identification, however, is extremely limited due to the many media, temperature conditions, and other environmental factors employed. Noteworthy papers of this nature are those of Rhoads (45) on Polyporus pargamenus Fr., White (59) on Fomes applanatus (Pers.) Wallr., Spaulding (50) on Lenzites saepiaria Wulf. ex Fr., Bayliss (2) on Polyporus versicolor Fr., Ward (58) on Stereum hirsutum Fr., White (60) on Corticium galactinum (Fr.) Burt, and Nobles and Nordin (42) on Corticium vellereum Ellis & Cragin.

2.2) Vegetative Hyphal Fusions

The first known reference to the occurrence of hyphal fusions or anastomoses is that of Brefeld (4), who recorded great numbers of hyphal fusions in the monosporous mycelia of various Coprini. Whether these fusions were of a sexual nature is not clear.

Marshall Ward (57) in 1888 recognized hyphal fusions between hyphae of a single culture of Botrytis. Ward reported an apparent attraction and deflection of the growing tips of hyphae destined to fuse. Rothert (47) noted hyphal fusions in Sclerotium hydrophilum Sacc. Falck (20) reported anastomoses as being characteristic of the primary and secondary mycelium of Lenzites abietina Fr. He illustrated hyphae of L. abietina present in the tracheids of Abies alba Mill. and depicts them joined in a network. Falck and Falck (21) also recorded hyphal fusions in the monosporous mycelium of Psalliota campestris Fr. Falck's 1909 reference to hyphal fusions in L. abietina is the first account of this phenomenon in the dikaryotic mycelium of the Hymenomycetes. Matsumoto (33) noted hyphal fusions in Rhizoctonia solani Kühn.

Buller (7) observed hyphal fusions in dikaryotic mycelium of Panus stypticus Fr. This reference is mentioned in greater detail in the review of literature concerning hyphal fusions, taxonomic speculation, and identification.

Burgeff (10) drew attention to the phenomenon of hyphal fusions in the Mucorales and pointed out the great similarity between parasitic and vegetative fusions in this group.

Buller (8) emphasized the importance of hyphal fusions in the social organization of the Hymenomycetes. Formation of hyphal fusions converts competition between several thalli into co-operation and thus aids in sporophore production. Buller records hyphal fusions in several species of Coprinus and in Panus stypticus Fr.

2.3) Action at a Distance and Fusion Formation.

De Bary (17) was the first investigator to recognize an apparent attraction between two approaching hyphae that were destined to fuse. The fungus studied was Pythium debaryanun Hesse and the resulting fusion was sexual in nature. The oogonium apparently stimulates neighbouring hyphae to send out antheridial branches. Ward (57), in his study of a Botrytis disease of lily, noted the attraction and mutual deflection of the growing tips of approaching hyphae. Ward recognized two types of reaction; stimulation of side branches in the case of two hyphae growing parallel, and stimulation of two growing tips to meet. Reinhardt (44) described and figured mutual attraction of approaching hyphae in various species of Sclerotinia.

Rothert (47), in discussing hyphal fusions in Sclerotium hydrophilum Sacc., reported that hyphae which unite are bent out of their direction of

growth and are apparently drawn together as if by the excretion of some stimulating substance.

Blakeslee (3), in his classic researches on sexual reproduction in the Mucorales, noted mutual attraction in approaching hyphae. He termed this phenomenon, "zygotactic".

Burgeff (10) in reporting on an investigation into the sexuality and parasitism in the Mucorales, coined the term "telemorphosis" to describe the induction of sexual hyphae by action at a distance through the air or substrate. The term "zygotropism" denoted the growth of a (+) and (-) zygophore toward each other, and "thigmorphosis", the swelling of the ends of the (+) and (-) zygophores once they had met. Burgeff postulated that volatile sexual substances were responsible for action at a distance. He also demonstrated that hyphae of host and parasite attract each other in the case of Parasitella simplex Bain and Chaetocladium brefeldii van Tiegh. & Le Mon.

Köhler (26) confirmed Ward's observation on action at a distance in the genus Sclerotium and suggested the use of Burgeff's terms "telemorphosis" and "zygotropism" in the higher fungi. He proposed the use of "telemorphosis" to denote the stimulation of one mycelium to produce side branches by another, and "zygotropism" to denote the curving together of two hyphae till they meet tip to tip. Buller (9) agreed with Köhler's proposed use of Burgeff's terminology. Regarding the method of fusion formation, Hein (24) observed that hyphae of Psalliota campestris apparently fused laterally by dissolution of the cell walls to form vascular elements of large diameter. Buller (9) claims that all fusions are actually end to end with regard to the approaching

hyphae. He enumerated four possible forms of fusion as follows: 1) hypha to hypha, 2) hypha to peg, 3) peg to peg, and 4) hook to peg (clamps). The term "peg" is here used to denote the short side branch put out to meet an approaching hyphal tip. Buller discounted Burgeff's theory of the excretion of some attractant by the approaching hyphae.

2.4) Hyphal fusions in Taxonomy and Identification.

Reinhardt (44) was apparently the first to consider hyphal fusion phenomena in taxonomic speculation. In three species of Sclerotinia, he observed that when two mycelia of the same species are paired in culture, they grow together and fuse smoothly. In pairings of different species, the fungi interfere with each other's growth. Hyphae of one colony penetrating the other are attacked and killed and fusions are never formed. Reinhardt cited this lack of fusion as evidence that the three organisms studied were distinct species.

Meyer (37) presented a list of fungi in which hyphal fusions occurred and discussed the physiological significance of the channels which hyphal fusions provide. He found no proof of fusions between hyphae of different species while con-specific hyphae often fused even if from different individuals.

Laibach (28), working with germ mycelia of several species of Septoria, reported hyphal fusions between isolates of the same strain but only bridges or contacts between different strains. Bridges but no fusions were also noted between what he considered to be two species, Septoria apii (Bri. & Cav.) Chester and S. petroselini Desm. Laibach suggested the possible future application of the hyphal fusion criterion in determining relationships between

forms of doubtful distinctness. In later work, Laibach (29) stated that no tendency to form hyphal fusions existed between Septoria humuli Westd. and S. aenotherae, and in 1928 (30), he reported intra-specific hyphal fusions in Leptosphaeria coniothyrium (Fuckel) Sacc. and in Monilia fructigena Pers.

Matsumoto (33) added to the growing body of evidence against inter-specific formation of hyphal fusions with his report of the regular and frequent occurrence of fusions between hyphae of the same strain of Rhizoctonia solani Kühn. Hyphal fusions were not produced regularly between different strains of R. solani. Forsteneicher (22), Matsumoto, Yamamoto, and Hirane (35), and Matsumoto and Yamamoto (34) also worked with R. solani and agreed with Matsumoto's original observations and in addition, reported no cases of inter-specific hyphal fusions between R. solani and Corticium stevensii Burt and Corticium koleroga (Cooke) Höhn.

Buller (7) reports the formation of hyphal fusions between dikaryotic mycelia of Panus stypticus Fr. isolated in Europe and North America. This information was regarded by Buller to substantiate the view that the two forms of P. stypticus which occur on opposite sides of the Atlantic are con-specific. This is the first reference to the use of hyphal fusions in dikaryotic mycelium as an aid in identification, and the first claim that such hyphal fusions occur only intra-specifically.

Davidson, Dowding, and Buller (16) working with dermatophytes reported hyphal fusions were formed abundantly in intra-specific pairing but were never produced in their inter-specific pairings. These authors suggest using the hyphal fusion technique in determining fungi in this difficult group. The unknown fungus could be paired against an identified culture collection

and the determination made on the presence or absence of fusions. De Cisneros (18) questioned the value of the fusion criterion in determining dermatophytes. This author found fusions occurring but rarely in these fungi and concluded the hyphal fusion method has only restricted application in their identification.

Vandendries (54), independently of Buller, made formal presentation of the theory that dikaryon hyphal fusions indicate a specific entity. In investigations on Coprinus micaceus Fr. Vandendries outlined the difficulty involved in using interfertility tests from single spore cultures as a means of identification. In the first place, single spore cultures are difficult to obtain if the fungus in question does not fruit in culture and secondly, many species produce spores that are difficult to germinate on artificial media. Vandendries' criticism of the single spore technique was that the unknown and the known culture with which it is paired may have the same factorial constitution and although belonging to the same species would not be fertile and hence give erroneous results. Vandendries states that monokaryon and dikaryon mycelia, no matter what their factorial make up, if they are con-specific will anastomose or form hyphal fusions. He claims further, that such anastomoses never occur between different species and therefore their formation indicates the cultures are con-specific. Vandendries and Brodie (54) reiterated the claim that vegetative hyphal fusions occurred only between pairings of the same species.

Melin (36) recommended the hyphal fusion method, on Buller's authority, as a method of identifying sterile mycorrhizal mycelia, which would presumably be dikaryotic. Lihnell (31), who was a pupil of Melin, used the hyphal fusion method on several root mycelia isolated from Juniperus communis L.

and other mycelia isolated by other workers from pine roots. On the basis of hyphal fusion formation, he concluded they all belong to the type Mycelium radicis atrovirens Melin which had been thought to include several species.

Robak (46) in an extensive investigation into the biology of seven common Norwegian wood rot fungi, demonstrated hyphal fusions between different dikaryotic isolates of the following organisms: Corticium evolvens Fr., Stereum purpureum (Pers.) Fr., Polyporus abietinus (Dicks.) Fr., Lenzites sepiaria (Wulf.) Fr. (rarely), Trametes odorata (Wulf.) Fr. and Trametes serialis Fr. No fusions were observed between different dikaryotic isolates of Stereum sanguinolentum (Alb. & Schw.) Fr.

In addition Robak made dikaryotic hyphal pairings between several different species whose distinctness appeared in doubt. Normal hyphal fusions were formed between the dikaryotic hyphae of Trametes odorata and T. americana Overh. and between dikaryotic hyphae of Stereum purpureum and S. rugosiusculum Berk. & Curt. Since the morphological similarities in the above two cases are great, Robak interprets the result as evidence for the con-specificity of the two fungi in both cases. Pairings between the fungi identified as S. sanguinolentum and S. rugosum (Pers.) Fr. irregularly produced peculiar fusions which Robak termed pathological, the hyphae breaking off very soon after fusion. While these two species are similar, they are widely accepted as distinct species.

Pairings of different forms of the variable species P. abietinus (Dicks.) Fr. gave interesting results. Isolates from the two extremes of the morphological variations would not fuse and displayed a form of antagonism in some instances, while the intermediates formed fusions with themselves and with the two extremes, (46).

Robak's conclusions are as follows: 1) if dikaryotic hyphae do fuse smoothly, they may be considered con-specific; 2) the fusion test will permit verification of con-specificity in some cases where the genetic constitution of the haploid prevents the use of the interfertility test; 3) when mycelial anastomoses do not form, this cannot be taken as evidence that the mycelia belong to different species; 4) in pairings of mycelia of different species, some phenomenon of antagonism may occur, but this may also occur between different pairs of the same species e.g. P. abietinus; 5) the fusion test is of supplementary value in taxonomic work, important only in cases of clearly positive fusions.

Cabral (11) published the results of some 800 pairings of dikaryotic mycelia of 21 species of Polyporaceae. This study was conducted in an effort to evaluate the dikaryotic hyphal fusion test for determining cultures of wood rot fungi. Only one case of apparent inter-specific fusion of dikaryotic mycelium was recorded, that between Leptoporus adustus (Willd.) Quél. and L. imberbis (Bull.) Quél. Correspondence with the supplier of the cultures of L. adustus indicated some doubt about the identification, with the distinct probability that they were incorrectly named and should have been referred to L. imberbis. Cabral was unable to demonstrate intra-specific dikaryotic hyphal fusions in two species, Polyporus sulphureus (Bull.) Fr. and Ungulina ochroleuca (Berk.) Pat. He concluded that the method can be considered of value but that negative results must be interpreted with care and more work done on the tendency of each species to form fusions under various environmental conditions such as temperature, pH, and culture medium.

The most recent reference to the hyphal fusion criterion in taxonomic

questions is that of Brodie (5). Formation of hyphal fusions between dikaryotic mycelium of a highly aberrant Cyathus and that of C. poeppigii Tul. is accepted as valid evidence that the aberrant culture is a form of the latter species.

2.5) Fungus Hybrids.

True experimental hybrids in the Hymenomycetes are apparently non-existent. The first reference is that of Vandendries (52) in which he reports a fertile pairing between monokaryons of Panaeolus campanulatus Fr. and Panaeolus fimicola (Fr.) Gillet. This report was revoked however by Vandendries (54) in 1933.

Routien (48) reported an intergeneric cross between Annelaria separata (Fr.) Karst. and Naucoria semiorbicularis (Fr.) Quél. but this occurred in only one pairing and the author was apparently not thoroughly convinced of the authenticity of the few clamp connections seen.

The reports of non-fertility in attempts at crossing various species of Hymenomycetes are frequent; Brunswick (6), Kniep (25), Vandendries (54), Mounce & Macrae (40), and others. The interfertility concept as a valid specific criterion is almost universally accepted in mycological circles at the present time.

Two papers on the general subject of hybrids in fungi should be noted. Moreau and Moreau (39) emphasized the difference between a mixed mycelium resulting from a vegetative hyphal fusion and the true hybrid resulting from a sexual fusion. These authors compared the former condition to a "graft hybrid" and rightly pointed out that it does not merit the term hybrid.

Winge (64), also drew attention to this distinction between vegetative and sexual fusions and termed the result of the former "dicaryophytic species hybrids" if occurring between different species.

Cabral (11) makes no such clear distinction in his summary of previous work on hybrids in the Hymenomycetes, and implies that the lack of true hybrids in this group is valid evidence favouring the hypothesis that vegetative fusions between species do not occur.

3. MATERIALS AND METHODS

3.1) Review of Previous Methods in Hyphal Anastomosis Work.

Dickinson (19) outlined a method for observing hyphal fusions between single sporidial hyphae in the smuts. A slice of agar on whose surface the two mycelia were growing was exposed to osmic acid fumes for 5 minutes to kill the hyphae. After drying in air for 15 - 30 minutes, the agar was inverted on a slide smeared with egg albumen and the latter promptly placed in strong Flemming's solution for one-half hour. The material was stained with Heidenhain's hematoxylin after which the agar was lifted off with a scalpel. The slide was then taken up through an alcohol series and mounted in the usual way.

Laibach (30) working with Leptosphaeria coniothyrium (Fuckel) Sacc. and Monilia fructigena Pers. emphasized that a scarcity of nutrient material seemed to stimulate the hyphae to fuse.

Köhler (27) in studying hyphal fusions in several Ascomycetes and Fungi Imperfecti, employed slides that had been dipped in melted agar. These

were inoculated with conidia of two different species with a small brush. In cases where the conidia were very similar some were treated with a mordant (1% tannin soln.) and then stained with distinctive basic dyes. Slides were kept on wet filter paper in petri dishes and a cover slip added for observation.

Davidson, Dowding & Buller (16) in studies on hyphal fusions in dermatophytes used a hanging drop of Sabouraud's medium in a Van Tieghem cell. Inoculations of small pinhead masses of mycelium of the fungi in question were made in pairs in the hanging drop of medium. Hyphal fusions were observed in a matter of five days between hyphae growing out from the hanging drop and adhering to the under side of the cover slip. Humidity was maintained in the cells by placing a few drops of sterile distilled water in the bottom of the glass ring.

Matsumoto, Yamamoto, & Hirane (35) employed a drop culture method very similar to that of Davidson, Dowding and Buller. They, however, substituted distilled water drops for the nutrient medium and specifically noted that hyphae from the advancing margin of the stock cultures (1-3 days old) were used in all their pairings. A modification of the technique by these authors is said to have facilitated the study of hyphal growth in detail. A loopful of 1.5% agar was spread over the surface of each cover glass and the pairings sown thereon instead of in the hanging drop. No mention is made of the distance between the pieces of inoculum after placing in the cells but fusions took place "about 10-12 hours after transfer" in these experiments. These authors tested the effect of prior growth on a series of synthetic agars on the tendency of Rhizoctonia solani to form hyphal fusions. They concluded that, for this particular fungus, the growth on differing media had

no effect on its tendency to form hyphal fusions.

Lihnell (31), in conducting experiments on the value of the hyphal anastomosis concept in identifying and segregating a group of root mycelia, devised a somewhat simplified method for bringing various pairs of inocula in close proximity for observing fusion of their hyphae. The mycelia to be tested were grown on tap water agar for several days and then two small cubes of agar containing the test mycelia were compressed between a cover glass and slide. These slides were then incubated in moist chambers and observed from time to time. Robak (46) experienced difficulty with this technique and concluded that a good deal of practice was required in order to have the test mycelia an appropriate distance apart after the agar had been compressed and crushed. Lihnell noted that in his experience, young and vigorous mycelia were less apt to fuse.

Robak (46) used a modification of the well known hanging drop method: "two small cubes taken from fresh tap water agar cultures were placed on the underside of the cover glass at a distance of 2-4 mm. "....." the cubes were placed on the cover glass without any other medium between them. Thus the hyphae had to meet on the glass wall". This statement is not perfectly clear but Robak was not writing in his native language and it can safely be assumed he meant that the hyphae would tend to meet on the lower surface of the cover glass. He goes on to point out one possible disadvantage of this method over that of Lihnell (31) in that aerial hyphae will usually escape examination. Robak, however, discounted this difficulty; apparently the fungi he studied produced very little aerial hyphae when grown on tap water agar.

Cabral (11) employed the Robak technique almost exclusively in his experiments on hyphal fusions between dikaryotic hyphae of various Polyporaceae. When pairing species of widely differing growth rates, Cabral found it necessary to place the slow growing fungus on the under surface of the cover slip several days in advance of the inoculum from the faster growing culture. This author offers evidence indicating that the culture medium on which the test fungi are grown immediately before pairing may have a marked effect on the tendency to fuse. Hyphal anastomoses were observed between different dikaryotic isolates of Merulius lacrymans (Wulf.) Fr. only when it had been grown on carrot agar prior to pairing.

3.2) Source of Cultures.

A total of 18 isolates of six species of Thelephoraceae was used. These originated from various parts of Canada and the United States and are being maintained in the stock culture collection of the Forest Biology Laboratory at Calgary, Alberta. The collection data on each culture are listed in Table I.

TABLE I

LIST OF CULTURES AND THEIR SOURCES

Culture number*	Fungus	Laboratory origin	Collector and locality	Host	Determination of culture	Source of culture
C 1	Corticium vellereum Ellis & Cragin	DAOM**	By E.G. Riley Lac du Bonnet, Man.	Ulmus pumila	M.K. Nobles	From sporophore
C 2	"	"	By V.J. Nordin Dorset, Ont.	Acer saccharum	"	From trunk rot by V.J. Nordin
C 3	"	"	By V.J. Nordin Dorset, Ont.	"	"	"
C 34	Stereum sanguinolentum Alb. & Schw. ex Fr.	DACFP***	By M.J. Hewitt Kananaskis, Alta.	Pinus contorta	"	From sporophore by W. Sutton
C 50	"	FPL****	By W.P.K. Findlay Locality unknown	Unknown	W.P.K. Findlay	By W.P.K. Findlay
C 78	"	DACFP	By D.E. Etheridge Luscar, Alta.	Picea engelmanni	M.K. Nobles	Polysporous culture by D.E. Etheridge
C 130	Peniophora aspera (Pers.) Sacc.	"	By V.J. Nordin Strachan, Alta.	Pinus contorta	R. Macrae	From sporophore by V.J. Nordin
C 154	"	DAOM	By ? Dorset, Ont.	Betula lutea	M.K. Nobles	Polysporous culture
C 153	"	"	By ? Cinema, B.C.	Populus trichocarpa	"	Polysporous culture

TABLE I (Cont'd.)

Culture number*	Fungus	Laboratory origin	Collector and locality	Host	Determination of culture	Source of culture
C 144	Corticium galactinum (Fr.) Burt	DAOM	R.W. Davidson Beltsville, Md., U.S.A.	Malus sp.	R.W. Davidson	Polysporous culture by R.W. Davidson
C 145	"	"	"	Cornus sp.	"	"
C 146	"	"	By ? Wilno, Ont.	Pinus resinosa	"	Polysporous culture
C 131	Peniophora gigantea Massee	DACFP	By E.J. Carmichael Strachan, Alta.	Pinus contorta	R.J. Bourchier	From wood by E.J. Carmichael
C 132	"	"	"	"	"	"
C 155	"	DAOM	By ? Seebe, Alta.	"	M.K. Nobles	Polysporous culture
C 162	Stereum purpureum Fr.	"	By ? Cinema, B.C.	Populus trichocarpa	"	Polysporous culture
C 163	"	"	By ? Cinema, B.C.	Log	"	Polysporous culture
C 164	"	"	By ? Devils Lake State Park, Wisc., U.S.A.	Alnus sp.	"	Polysporous culture

* Stock culture collection, Forest Biology Laboratory, Calgary, Alberta.
** Mycological herbarium, Canada Department of Agriculture, Ottawa, Ontario (Dr. M.K. Nobles).
*** Mycological herbarium, Forest Biology Laboratory, Calgary, Alberta.
**** Forest Products Laboratory, Ottawa, Ontario.

3.3) Culture Media.

The stock culture collection at the Calgary Forest Biology Laboratory is carried on a malt agar prepared according to the following formula:

Difco powdered malt extract broth --------- 20 gm.

Difco-Bacto agar ---------- 20 gm.

Distilled water ---------- 1000 c.c.

Prior to pairing trials, all fungi were transferred from stock and grown for one week at 20° C. on petri plates containing 20 c.c. of the above malt agar.

The three culture media used to test the effect of different media on the formation of hyphal fusions were prepared according to the following formulae:

Malt agar - Identical to malt agar above

Potato Dextrose Agar

Difco-Bacto potato dextrose agar --------- 39 gm.

Distilled water ---------- 1000 c.c.

Carrot Dextrose Agar

Difco-Bacto dextrose ---------- 20 gm.

Difco-Bacto agar ---------- 20 gm.

Carrot extract from steaming 200 gm. sliced carrots in 500 c.c. distilled water for ½ hour----500 c.c.

Distilled water ---------- 500 c.c.

In testing the effects of the pH reaction of the culture media on the formation of hyphal fusions, the pH of equal aliquots of each of the four media

was adjusted to the pH values of 3.5, 5.0, 6.5, 8.0 plus or minus 0.1 pH unit using N/10 HCl or NaOH as required. To these adjusted portions of media, buffer solutions made up of varying proportions of sterile M/3 H_3PO_4, M/3 KH_2PO_4 and M/3 K_2HPO_4 solutions were added as outlined by Wolpert (62). Twenty per cent of the final medium in each case was buffer.

The pH values of the media were determined with a Beckman pH meter. The 3 ml. test portions were allowed to cool to 40° C. before pH readings were taken. Temperatures were checked with a thermocouple and Rubicon pyrometer.

3.4) Cultural Conditions.

The stock culture collection at the Calgary laboratory is maintained in a large refrigerator at a temperature of approximately 4° C.

In the experiments on the effect of temperature on hyphal fusions formation, the constant temperature cabinets, designed to maintain a specific temperature $\pm\frac{1}{2}$° C., were set at the following temperatures: 20° C., 25° C., and 30° C. To ensure that no unnoticed variation in temperatures occurred, a recording thermograph was placed in each cabinet.

3.5) Selection of Pairing Technique for Present Work.

In selecting and perfecting techniques for observing hyphal fusions in the Thelephoraceae, one or more of the six test fungi were utilized in evaluating three methods published by Robak (46), by Lihnell (31), and by Davidson, Dowding, and Buller (16).

Davidson, Dowding, and Buller used a hanging drop of plain agar in a Van Tieghem cell with a small amount of water in the bottom of the cell

to provide humid conditions. Twenty-one pairings of this type were made with four isolates of Corticium vellereum grown on fresh 2% malt agar plates for approximately two weeks. The cells were incubated at a constant temperature of 20° C. In view of the lack of published information on the temperature optimum for C. vellereum, 20° C. was chosen as a likely suitable temperature for growth of the fungus. Reports on temperature relations for other Thelephoraceae (12) indicate that many of this group show good growth at or near this temperature.

Corticium vellereum, produced good growth in the plain agar drop, but the drop made observation exceedingly difficult. Only after the agar drop had become accidently dried in one or two cells was it possible to focus on the hyphae between the two pieces of inocula with even the high dry objective. If one waited until hyphae had grown out beyond the agar drop, it was then possible to use the oil immersion objective and pass positive judgement on the presence of fusions. To trace the hyphae back to the inoculum from fusions outside the drop was impossible, however, and one could not say whether fusions were occurring between the two pieces of inocula or only amongst hyphae of one piece of inoculum. For these reasons the method was rejected.

Lihnell's method of crushing two small pieces of agar containing the inocula between cover glass and slide, while theoretically simple, proved difficult in practice. The two small agar blocks either shot out from under the cover glass or were forced together when the pressure was applied. Perhaps if the agar had been less solid, better results would have been obtained. The writer, however, is inclined to agree with Cabral (11) that the method requires

considerable practice and it was decided to reject it on these grounds.

A refinement of Robak's pairing method was selected for use in the present work. Pieces of inoculum of uniform size were cut from the advancing zone of week old petri plate cultures using a loop of flattened chromel wire. Two pieces of inoculum were cut out at a time, the second lifting the first out of the wire loop. It was then a simple matter to slide the first off the second and to place it on the lower surface of a cover slip with the surface mycelium pressed against the glass. Pieces of inocula were placed approximately one mm. apart on the cover glass. Humidity was maintained by placing a small amount of sterile distilled water in the bottom of the glass ring. Hyphae grew out from the pieces of inoculum and adhered to the cover slip facilitating observation with the high dry or oil immersion objective.

4. THE MICROSCOPIC APPEARANCE OF HYPHAL FUSIONS

The oil immersion objective was required in most cases to determine whether hyphal fusion had actually occurred between contacting hyphae. With the particular microscope employed this set up provided a magnification of 2000X. At this magnification, cellular contents could occasionally be discovered passing through hyphal fusions.

Several basic types of hyphal fusion were noted. In general they conformed to the classification of fusion types by Buller (9) with one notable exception. Buller's statement that all fusions were actually end to end with regard to the approaching hyphae was not corroborated.

The most common type of fusion observed in C. vellereum is illustrated

in figures 1 to 7. This corresponds to none of Buller's types. In this case, a growing hyphal tip approaches the side of another hypha and apparently fuses directly with the side wall of the latter. In this type of fusion, there is no evidence whatsoever of the production of a small peg from the side wall of the hypha as outlined by Buller (9).

A type of fusion corresponding to Buller's hypha to hypha is illustrated in figures 8 and 9. Two approaching hyphae meet tip to tip and a hyphal fusion results.

A possible peg to peg type of fusion is illustrated in figure 10. In this type, according to Buller, fusion is produced between two short pegs growing out from two closely lying hyphae. Fusion is said to be tip to tip. The fusion illustrated in figure 10 does not appear to be tip to tip, rather it would seem that fusion has taken place between the tip of one short peg and the side of the other.

Figures 11 and 12 illustrate the appearance of a hyphal contact where no fusion has occurred. The upper hypha has grown against the lower and been deflected to grow parallel to the latter.

5. THE TEST FOR OCCURRENCE OF HYPHAL FUSIONS IN THE THELEPHORACEAE

5.1) Methods.

The three isolates of each fungus listed in Table I were grown on malt agar plates at 20° C. for one week and then paired according to the modified Robak technique outlined above. In this test only intra-specific pairings were made, the three isolates of each species being paired in the

three ways possible. Each pairing was made nine times. These were then divided into three groups of three, with each group incubated at 20° C., 25° C., and 30° C., respectively.

Observations were made daily for a period of two weeks with the high dry and usually the oil immersion objectives of the microscope. Many of the cells were discarded as early as seven days after the experiment was started due to the hyphae becoming too dense and confused in the region between the pieces of inoculum.

5.2) Results.

Hyphal fusions were recorded at least once for five of the six fungi. Peniophora gigantea was the only species that did not produce any visible fusions in the two week period. The results are summarized in Table II.

TABLE II

OCCURRENCE OF HYPHAL FUSIONS BETWEEN DIKARYOTIC HYPHAL OF SEVERAL SPECIES OF THELEPHORACEAE

Fungus	Total number of cells out of 9 possible in which hyphal fusions were recorded			Average Elapsed time until fusion noted (Days)		
	20° C	25° C	30° C	20° C	25° C	30° C
Stereum sanguinolentum	0	0	1	-	-	7
Stereum purpureum	1	0	1	16	-	11
Peniophora aspera	5	5	5	5.7	8.2	4.4
Peniophora gigantea	0	0	0	-	-	-
Corticium vellereum	6	5	5	6.3	4.6	2.0
Corticium galactinum	2	0	2	3.5	-	5.5

It will be noted that temperature apparently has little or no effect on the total number of cells in which fusions were noted but seems to have considerable effect on the rapidity of fusion formation. The significance of the temperature effect on rate of fusion formation in Corticium vellereum and Peniophora aspera was examined and found to be significant at the 5% level for C. vellereum and approaching the 5% level for P. aspera.

6. THE TEST TO DETERMINE THE EFFECT OF TEMPERATURE, pH OF MEDIUM, AND MEDIUM FORMULA ON HYPHAL FUSION FORMATION

6.1) Materials and Methods.

Two isolates of Corticium vellereum were used in this experiment. Since the objective was to determine the effect of the three variables on hyphal fusion, it was originally decided to employ only one isolate (C 3) of C. vellereum in order to do away with any physiological variation. The second isolate (C 2) was included, however, in order to give some indication of the variation that could be expected within a given species. The experiment was designed for one isolate and simply repeated for the second isolate. Pairings were made between different pieces of inoculum of the same isolate only. The isolates C 2 and C 3 were grown on malt agar plates for one week at 20° C. to provide a sufficient supply of inoculum and in an effort to give the two isolates a similar recent history. Plates of the three culture media under test, viz., carrot dextrose agar, potato dextrose agar, and malt agar, were then prepared according to the formulae given under the materials heading. An equal number of plates of each medium was then adjusted to three pH values, 5.0, 6.5, and 8.0, after the method of Wolpert. Inoculum from the week old malt plates was then placed in the plates of various media and pH and incubated

at 20° C. for one week. These plates were the source of inoculum for the cells. Three cells at each pH and of each media were inoculated with the C 2 strain and three with the C 3 strain. This gave one cell of each medium at each pH, incubated at each of the three temperatures, 20° C., 25° C., and 30° C., for both the C 2 and C 3 strains of the test fungus.

The trial run was made with two pieces of inoculum of the same isolate placed in the cells and observed daily. A sample of 20 hyphal contacts was selected at random by moving the oil immersion objective about in the zone where the hyphae from the two pieces of inoculum were meeting. No tallies were made until the hyphae were coming into contact and only those tallies where 20 contacts were seen were included in the data.

Considerable difficulty was encountered using two pieces of inoculum. Once contact was made between approaching hyphae, the situation quickly became confused, making it nearly impossible to identify hyphae and actually determine whether the junction under observation was a branch or a fusion. The first fusions were easily picked out but getting a full sample of twenty proved troublesome. For this reason, it was decided to place only one piece of inoculum in each cell and count the number of contacts and fusions between the hyphae radiating from this single source. When this was done, little difficulty was experienced in obtaining the required 20 contacts because, as time went on, the hyphae tended to thin out rather than become closely meshed. Since the two pieces of inoculum were from the same isolate in the trial run, there is little basic difference between counting contacts and fusions between two pieces of inoculum and counting those produced from hyphae radiating from one piece of inoculum.

The experiment was set up as a factorial design with three replicates. Unfortunately, it was physically impossible to carry out the three replicates concurrently. It was felt that the examination of a series of cells should be completed in, at most, one day so that all cells might be in a similar state of development at each examination. Since each replicate contained one hundred and twenty cells and took approximately ten hours to examine, it was not feasible to run the three replicates simultaneously. The media for the three replicates were made up fresh each time from the same batches of dry materials in order to have them closely comparable.

The sample size of 20 contacts was also set on physical limitations. Experience showed that if the cells were maintained for longer than one week, excessive loss due to dehydration or contamination resulted. Since two counts well separated in time were required to show up the possible presence of a time factor, a compromise had to be made between sample size and the time between counts. The cells were prepared on a Monday and by Wednesday 20 contacts could be recorded in most cells. Counting on Wednesday allowed 48 hours to elapse before the second count was made the following Friday. This length of time was regarded as minimum. Further, the time required to count 20 contacts in 128 cells was around ten hours of steady microscopic examination, about the limit of accurate observation on the part of the writer.

The variability in different numbers of samples of twenty hyphal contacts was determined. Ten cells were inoculated from malt agar cultures of Corticium vellereum and incubated at 20° C. for one week. Three samples of 20 hyphal contacts from each cell were tallied for the presence or absence of fusions. A total of 30 samples of size 20 were examined; this approxmiates

the 27 cells used in a majority of the analyses. The mean and standard error were determined and found to be 1.10 and 0.17 respectively. Since the mean, plus or minus two standard errors gives the limits 0.76 and 1.44, it can be safely concluded that the variability of this size sample was within acceptable limits.

Fifteen samples of 20 contacts were selected at random from the above data and the mean and standard error determined. Results showed that samples of this size displayed excessive variability. Consequently results based on samples of this general size or smaller must be accepted with caution. This applies to the analysis on the effect of pH on hyphal fusion formation.

6.2) Results.

Unfortunately, a sufficient number of cells were lost through drying or through contamination so that it was impossible to analyse the data statistically as originally planned. Instead, sub-analyses were carried out employing all the available data for each comparison. The loss of cells also made it impossible to examine the data for relevant interactions between the various factors.

In comparing the effects of the three media on hyphal fusion formation, the F test of the Wednesday readings of the C 3 isolate using 540 contacts or 27 cells in the three replicates failed to show any significant differences in the number of fusions formed. The Friday readings, however, based on the same number of cells and contacts, showed a significant effect at the 5% level due to culture medium differences. In these data, the potato dextrose agar produced roughly twice the number of fusions that developed in

carrot dextrose or malt agar. P.D.A. also gave superior results in the Wednesday readings but the differences were not so marked.

The effect of culture medium differences on the C 2 isolate was also examined with the F test. No significant differences were shown either in the Wednesday or Friday readings. The former was based on 360 hyphal contacts or 18 cells and the latter on 180 contacts or 9 cells only. These small samples perhaps account for the lack of significant results. In both cases, however, potato dextrose agar produced the greatest number of fusions.

Table III summarizes the effect of the three culture media on hyphal fusion formation in two isolates of Corticium vellereum. It is worth noting that, without exception, potato dextrose agar produced superior results while malt agar and carrot dextrose agar were more similar in their effects.

TABLE III

THE EFFECT OF THREE CULTURE MEDIA
ON HYPHAL FUSION FORMATION IN THREE ISOLATES OF C. VELLEREUM

Strain	Time	P.D.A.	C.D.A.	Malt	Basis	F test Results
C 2	Wed. Readings	27	12	8	360 contacts 18 cells	Not significant
	Fri. Readings	12	5	10	180 contacts 9 cells	Not significant
C 3	Wed. Readings	40	15	.31	540 contacts 27 cells	Approaching the 5% level
	Fri. Readings	53	26	24	540 contacts 27 cells	Significant at 5% level

In examining the effect of three temperatures (20° C., 25° C., 30° C.)

on the formation of hyphal fusions, no significant effects were shown by the F test. This result is felt to be conclusive since the sample size was adequate in all cases. Three analyses were made, one using Wednesday readings of the C 2 isolate based on examination of 540 contacts and 27 cells; the other two were the Wednesday and Friday readings of the C 3 isolate, the former based on 540 contacts and 27 cells while the latter was based on 1080 contacts and 54 cells. Due to missing data from contamination it was impossible to perform an analysis of the Friday readings of the C 2 isolate. In addition to the lack of statistical significance, no consistent pattern could be discerned in the tables comparing the numbers of hyphal fusions obtained at different temperatures. Table IV gives a summary of the data.

TABLE IV

THE EFFECT OF THREE TEMPERATURES ON HYPHAL FUSION FORMATION IN TWO ISOLATES OF CORTICIUM VELLEREUM

Strain	Time	20° C.	25° C.	30° C.	Basis	F. Test Results
C 2	Wed. Readings	20	30	27	540 contacts 27 cells	Not significant
	Fri. Readings	Not available in all three replicates due to contamination				
C 3	Wed. Readings	17	36	16	540 contacts 27 cells	Not significant
	Fri. Readings	67	65	83	1080 contacts 54 cells	Not significant

The effect of the three pH values on hyphal fusion formation in Corticium vellereum was not significant in these experiments (Table V). The

Wednesday and Friday readings of the C 3 isolate, both based on 360 contacts of 18 cells, failed to show any significance. Similar results were obtained with the data for the C 2 isolates. In the latter case the Wednesday readings were based on 180 contacts and only 9 cells and no analysis was possible for other Friday readings due to lack of data caused by contaminated cells.

TABLE V

THE EFFECT OF CULTURE MEDIA OF THREE DIFFERENT pH VALUES ON HYPHAL FUSION FORMATION IN 2 ISOLATES OF CORTICIUM VELLEREUM

Strain	Time	pH 5.0	pH 6.5	pH 8.0	Basis	F Test Results
C 2	Wed. Readings	13	16	13	180 contacts 9 cells	Not significant
	Fri. Readings	-	-	-		Not significant
C 3	Wed. Readings	21	19	14	360 contacts 18 cells	Not significant
	Fri. Readings	16	24	19	360 contacts 18 cells	Not significant

The data were also examined with the F test for differences in the behavior of the C 2 and C 3 isolates of the test organism. The results are outlined in Table VI.

Although the F test revealed no significant differences between the two strains, the C 3 isolate produced slightly more fusions on both Wednesday and Friday. However, this relationship was not uniform throughout all replicates.

TABLE VI

COMPARISON OF C 2 AND C 3 ISOLATES OF CORTICIUM VELLEREUM IN THEIR TENDENCY TO FORM HYPHAL FUSIONS

Wednesday Readings					Friday Readings				
lture ium	C 2	C 3	Basis	F Test Results	Culture Medium	C 2	C 3	Basis	F Test Results
D.A.	36	46	480 contacts 24 cells	Not Sig.	P.D.A.	20	19	120 contacts 6 cells	Not Sig.
D.A.	49	50	960 contacts 48 cells	Not Sig.	C.D.A.	47	49	600 contacts 30 cells	Not Sig.
lt	4	4	120 contacts 6 cells	Not Sig.	Malt	24	29	360 contacts 18 cells	Not Sig.

7. THE TEST TO DETERMINE THE EFFECT OF MEDIUM FORMULA ON GROWTH RATE OF CORTICIUM VELLEREUM

Of the three factors studied for their effect on hyphal fusion formation, only medium formula produced an effect detectable in our experiments. As outlined previously, potato dextrose agar was superior to carrot dextrose agar as regards numbers of fusions formed and these were both superior to malt agar. It was considered of interest to compare the growth rates of the test organism, C. vellereum on the three media.

7.1) Materials and Methods.

The three media were prepared and adjusted to a uniform pH of 4.5. Ten petri plates of each media were inoculated with the C 2 strain of Corticium vellereum and ten with the C 3 strain and incubated at 20° C. The inoculum came from malt plates grown for 6 days at 20° C.

On the fourth day after inoculation, the growth on the plates of various media was measured in cms. along two radii at right angles. These measurements were repeated along the same radii on the seventh and twelfth days. The experiment was replicated three times, the replicates running concurrently in the same temperature cabinet.

7.2) Results.

Table VII gives the mean diameters of the ten plates measured for each isolate and culture medium. It will be noted that the carrot dextrose agar medium gave consistently better growth than the other two media, while the malt produced the least growth.

These data were examined with the F test and shown to be highly significant (better than the 1% level) as regards the effect of the three culture media on growth.

TABLE VII

THE AVERAGE DIAMETER IN CENTIMETRES OF 10 PETRI PLATES EACH OF THE C 2 AND C 3 STRAIN OF CORTICIUM VELLEREUM GROWING ON VARIOUS CULTURE MEDIA

		C 2			C 3		
plicate	Medium	Days after inoculation			Days after inoculation		
		4	7	12	4	7	12
	P.D.A.	1.99	3.61	7.16	2.36	4.56	8.15
1	C.D.A.	2.57	4.74	8.45	2.68	4.71	8.04
	Malt	1.58	2.72	5.32	2.06	3.99	7.33
	P.D.A.	1.99	3.64	7.05	2.31	4.14	7.42
2	C.D.A.	2.42	4.40	8.15	2.47	4.64	7.98
	Malt	1.87	3.08	5.34	2.10	3.92	7.22
	P.D.A.	1.72	3.36	6.98	2.29	4.14	7.40
3	C.D.A.	2.40	4.21	7.84	2.56	4.63	8.07
	Malt	1.54	2.60	5.24	1.88	3.59	6.78

It is evident that the culture medium producing the greatest number of hyphal fusions in these particular strains of C. vellereum is not the most suitable for growth of the fungus.

The data in Table VII were also analysed for differences between the C 2 and C 3 strains. On malt agar, the difference in growth rate between the two isolates becomes more marked with time. The four day figures reveal a difference significant at the 5% level while the 12 day data show a

difference significant at the 1% level. On the other hand, when the two isolates are grown on carrot dextrose agar or potato dextrose agar, the differences become less marked in time. The F test revealed no significant differences either in the four day data or the 12 day data between C 2 and C 3 strains when grown on carrot dextrose agar (the best medium of the three for growth). On potato dextrose agar a difference significant at the 5% level was revealed when the four day data were analysed but no significant differences occurred in the 12 day data.

8. DISCUSSION

8.1) Occurrence of Hyphal Fusions in the Thelephoraceae.

As expected, hyphal fusions do occur in members of the Thelephoraceae although the tendency to form fusions varies from species to species. The lack of fusions reported for Peniophora gigantea is probably the result of unfavourable conditions of the medium. The influence of culture media on the tendency to form fusions is demonstrated in this paper. It is, in fact, surprising that fusions were recorded in so many of the test species when only one culture medium (malt agar) was used. It is probable that hyphal fusions can be produced experimentally in P. gigantea and in other Thelephoraceae if the environmental conditions, particularly nutrient supply, are favourable.

The effect of temperature on the timing of hyphal fusion is not surprising. The effect of this factor on fungus growth rates is well known. The results for Peniophora aspera and Corticium vellereum outlined in Table II simply indicate that, at 20° C. for the latter and at 25° C. for the former, growth was more rapid, the hyphae contacted each other earlier, and hence

fusions were noted in a shorter period at these temperatures.

8.2) The Effect of pH and Formula of Medium, and of Temperature on Hyphal Fusion Formation.

While pH was not shown to produce significant results on hyphal fusion formation in these experiments, it perhaps does have some effect on this phenomenon beyond the precision of this experiment. The results indicate the unimportance of temperature in hyphal fusion formation. There is the possibility that both these factors might have a more marked effect on hyphal fusion formation at the extreme tolerances of the fungus. This contingency was not explored. Since the potential result of the work is the development of a quick identification technique for cultures, there seemed to be little point in working at environmental extremes where growth would be so slow that the advantage of rapid identification would be lost.

Culture medium formula has an important effect on hyphal fusions. Not only was it shown to be significant in one of the tests, but the same pattern was displayed in the other tests pointing to the superiority of potato dextrose agar. Whether this medium lacks some nutrient factor and promotes fusion in this manner or contains some factor especially favourable to hyphal fusion formation is open to conjecture. The fact that potato dextrose agar was inferior to carrot dextrose agar as a medium for growth may indicate that it is a lack of some factor in the former medium that aids in fusion. The hypothesis of nutrient deficiency stimulating fusion is supported in the literature by Laibach (1928) who claimed greater fusion formation in Leptospharia coniothyrium and Monilia fructigena when they were grown on agar poor in nutrients. The findings presented here regarding the importance of culture medium in hyphal fusion work, are compatible with those of Cabral

who was able to produce hyphal fusions between different dikaryotic isolates of Merulius lacrymans only when it had been grown on carrot agar prior to testing.

These data suggest the hypothesis that different isolates of the same species have similar hyphal fusion behavior. However, because only two isolates were compared, this hypothesis must be regarded with extreme caution pending tests with numerous strains.

While the data point to a similarity between the two strains in fusion tendencies, their growth rates diverged widely. On malt agar, a marked difference in growth rates of the C 2 and C 3 isolates was shown. On potato dextrose agar, the initial difference in growth rates of the two strains may be explained as an effect persisting from the malt agar on which they had been previously grown. However, any such persistent effect of the malt was not shown when the two strains were transferred to carrot dextrose agar.

8.3) Use of Hyphal Fusions in Culture Identification Work.

The major drawback of the hyphal fusion technique for identifying cultures of fungi has been the uncertainty involved in negative results. The fact that fusions are not formed does not constitute proof that the two isolates are not con-specific because, on several occasions, investigators have been unable to produce fusions between known con-specific isolates (46, 11). Evidence presented in this paper offers some assurance of circumventing this major obstacle. The fact that culture medium is shown to be an important factor in fusion formations leads to the hope that individual optimum

conditions for hyphal fusion in a large group of fungi can be determined. This would reduce substantially the frequency of non-fusion between members of the same species, and in turn increase the confidence that could be placed in negative results.

Much work remains to be done. Future studies should be designed to yield more precise information on the variability of different isolates of a fungus species in hyphal fusion behaviour. In addition, the optimum culture conditions for fusion in many species must be determined before routine identification by the hyphal fusion technique becomes feasible.

9. SUMMARY

Because of the lengthy procedures and experience required to identify cultures of wood rot fungi, a project was initiated in 1954 to gain more information on the practicality of the hyphal fusion technique of identification.

Hyphal fusions occurred between different isolates of the following species: Corticium vellereum, C. galactinum, Peniophora aspera, Stereum sanguinolentum, S. purpureum. No fusions were found between different isolates of Peniophora gigantea.

The appearance of hyphal fusions in C. vellereum is outlined and the various types observed are compared to Buller's classification of hyphal fusion types. Buller's claim that all fusions form between the tips of approaching hyphae was not confirmed. Fusions were observed between hyphal tip and side wall.

Culture medium formula was found to have significant effects on the number of fusions formed within two strains of C. vellereum when the data were statistically examined. Potato dextrose agar consistently produced greater numbers of fusions in this species.

Culture medium has a highly significant effect on the growth rate of C. vellereum and the medium producing the greatest growth, carrot dextrose agar, differed from that producing the greatest numbers of fusions.

No significant differences due to temperature or pH variations were revealed.

No significant difference in hyphal fusion behaviour was detected between two strains of C. vellereum.

The potential usefulness of the phenomenon of hyphal fusions in culture identification is discussed. There is some hope of developing the hyphal fusion technique for practical identification work.

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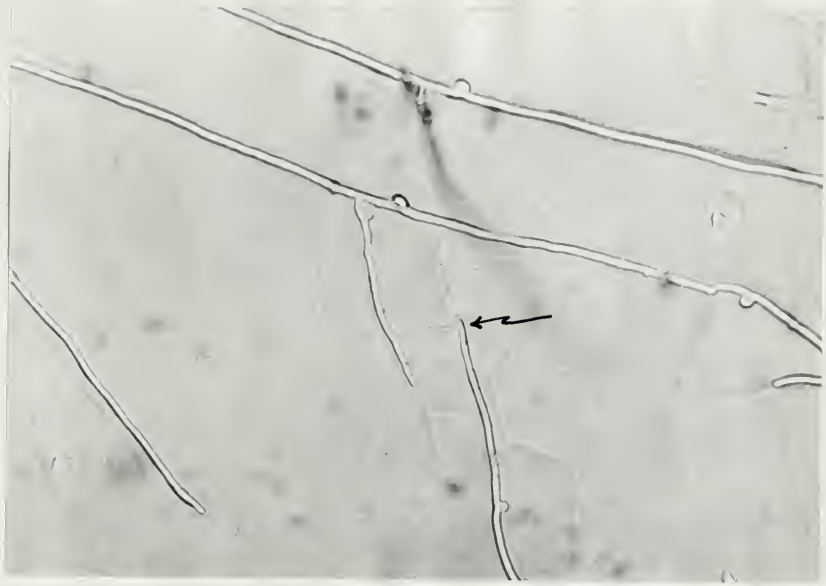
12. PLATES

PLATE I

Fig. 1. A hyphal fusion of the tip to side type. Note the disintegrating hypha on the right above the fusion. Approx. 600X

Fig. 2. A photographic enlargement of the above fusion. Approx. 1200X



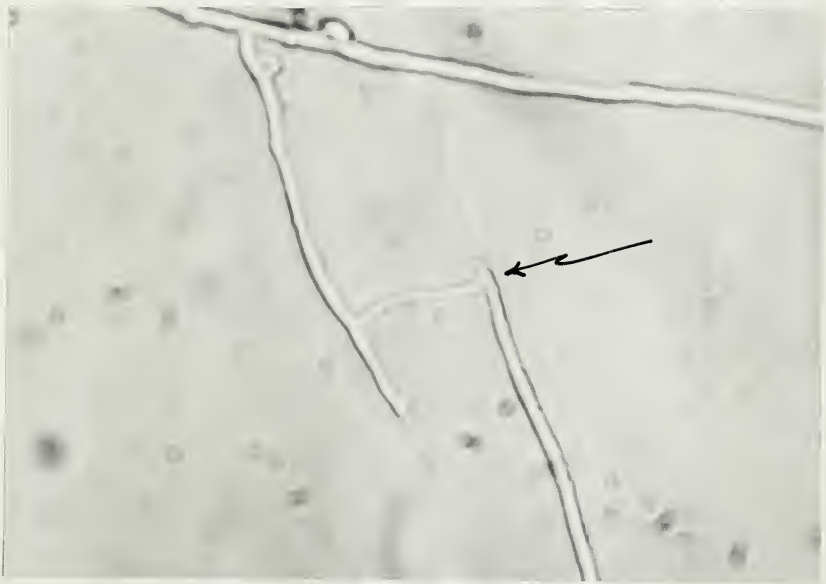
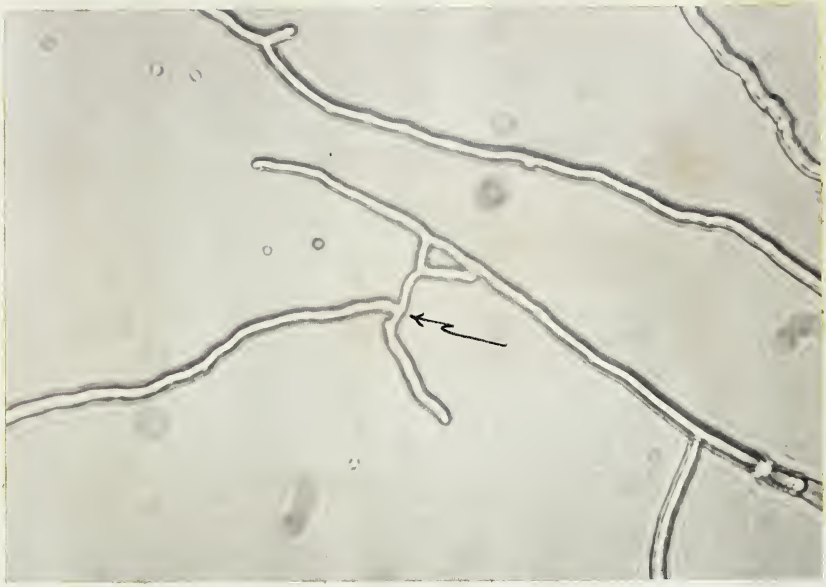


PLATE II

Fig. 3. A hyphal fusion of the tip to side type. Approx. 1200X

Fig. 4. A photographic enlargement of the above. The actual break in the cell walls can be discerned. Approx. 2500X



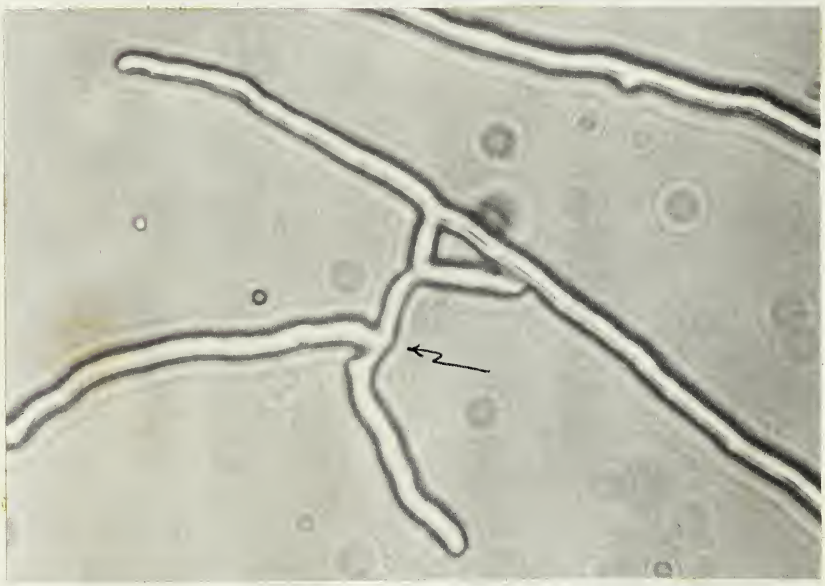
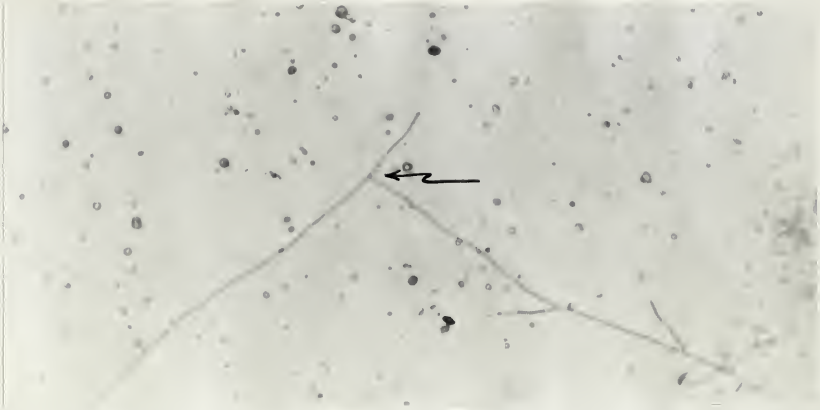


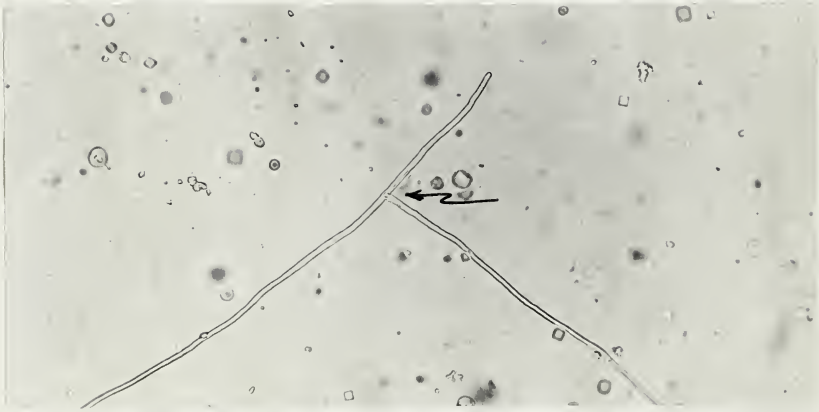
PLATE III

Fig. 5. Appearance of two hyphae that have formed a tip to side hyphal fusion. Approx. 200X

Fig. 6. The above fusion in greater detail. Approx. 400X

Fig. 7. The same fusion as illustrated in Figs. 5 and 6, but photographed at a high enough magnification to show the actual break in the cell walls. Approx. 1000X





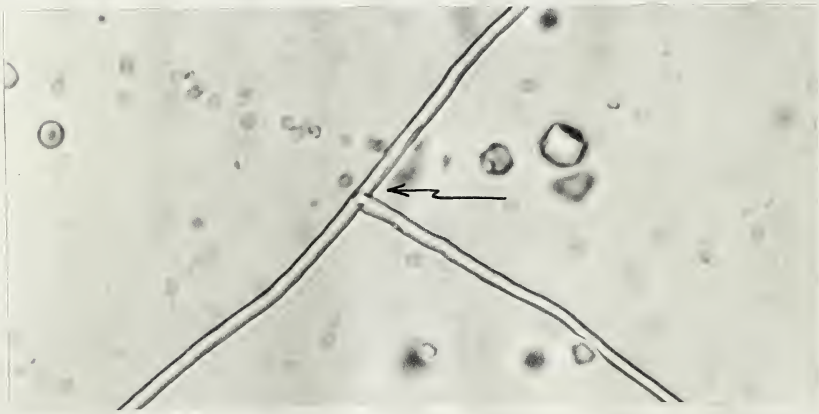


PLATE IV

Fig. 8. A hyphal fusion that possibly formed by two hyphae meeting tip to tip. Approx. 960X

Fig. 9. The same fusion as above in greater detail. Approx. 1500X

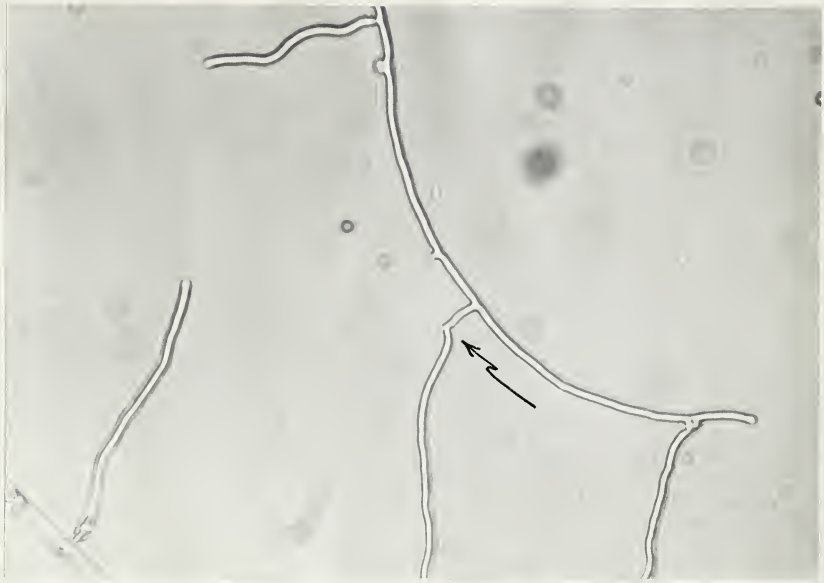


PLATE V

Fig. 10. A hyphal fusion formed between two short side branches. Note the opening in the cell walls. Approx. 2000X

PLATE VI

Fig. 11. A hyphal contact. Note how the upper hyphae has contacted the lower and been deflected to the right. Approx. 400X

Fig. 12. The same hyphal contact as above at a greater magnification. Note the condensed moisture on either side of the hyphae. Approx. 1600X